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Antitubercular agents. Part 2: New thiolactomycin analogues active against *Mycobacterium tuberculosis*

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Abstract—Structurally modified analogues of naturally occurring antibiotic thiolactomycin, substituted at 4-position of the thiolactone ring have been prepared and evaluated for their antitubercular activity. Some of the compounds have exhibited potential activity against *Mycobacterium tuberculosis*.

Mycobacterium tuberculosis, which causes tuberculosis, is the greatest single infection responsible for mortality worldwide, killing roughly two million people annually.[1] Current estimates indicate that one-third of world's population is with latent *M. tuberculosis*.[2] Despite the fact that it is treatable and preventable, the disease has been spreading at a steady rate over the past decade.[3] The emergence of bacterial resistance to the multidrug resistant TB (MDRTB) of existing antitubercular agents has become a significant concern for the effective treatment of tuberculosis. The determination of whole-genome sequence of *M. tuberculosis*[4] has pinpointed drug targets that have potential for improved therapy.[5]

Thiolactomycin (TLM, **1**) that has a thiolactone ring system is an antibiotic obtained from the fermentation broth of a strain of actinomycetes, which was identified as species of *Nocradia*,[6] is active against in vitro Gram positive, Gram negative and anaerobic bacteria. Thiolactomycin is a unique molecule that exhibits selective activity against only the dissociable type II fatty acid synthesase (FAS) enzymes.[7] TLM is a reversible inhibitor[7a] of β-ketoacyl synthesase (KAS) of bacterial FAS systems including KAS I-III and acetyl coenzyme A (CoA): ACP transacylase activities in vitro and in vivo in *E. coli*.[8] Crystal structure of KAS I (Fab B from *E. coli*) bound with TLM reveals the essential enzyme–ligand binding interactions and establish the existence of hydrophobic and pantetheine binding pockets that are both unoptimally filled.[9] Previous studies reveal that a number of TLM analogues with aliphatic side chains at 5-position of thiolactone ring have shown significant enhancement of activity against mycolate synthesis.[10,11]

Recently, a number of biphenyl based[12] and acetylene based[13] TLM analogues have been synthesized that exhibit significant activity against mtFabH fatty acid condensing enzyme. These results from earlier studies illustrate the importance of side-chain structure in TLM analogues. However, no efforts have been made for preparing analogues by substituting the 4-position of the thiolactone ring. Studies have also been carried out in this laboratory for the development of antitubercular compounds based on other class of prototypes.[14] Therefore, in the present investigation an attempt has been made to prepare 4-substituted thiolactones and interestingly some of these analogues have exhibited promising in vitro antitubercular activity.

Thiolactone ring **2** which is the key intermediate for the preparation of such analogues has been synthesized by employing the procedure of Wang and Salvino.[15] This method involves Claisen self ester condensation of methyl propionate **3** using KH to yield β-keto ester **4**. The selective bromination of **4** followed by nucleophilic substitution of bromine with thioacetic acid gives thio

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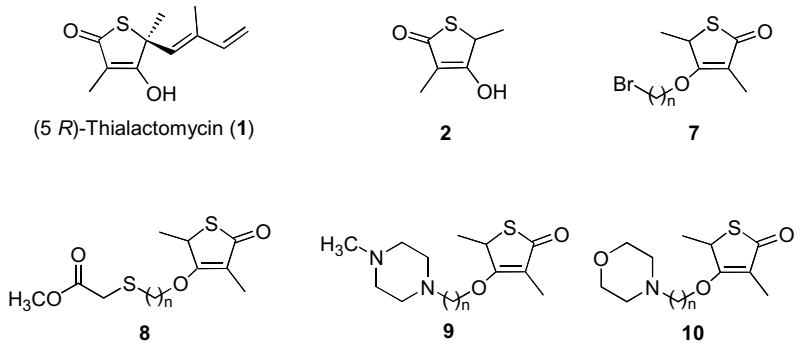
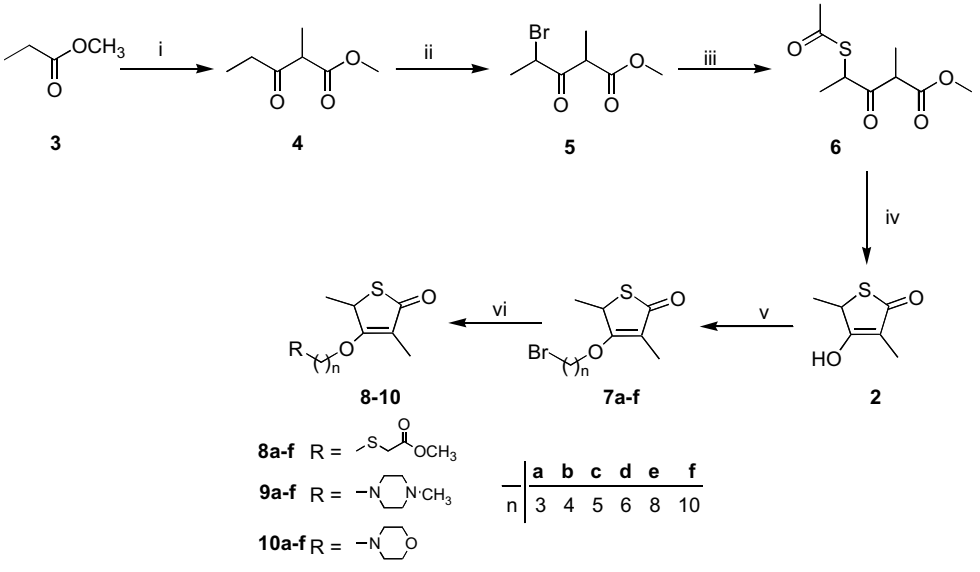


ester **6**, and finally cyclization to afford the 4-hydroxy thiolactone **2**. The required analogues have been prepared by the etherification of 4-hydroxy group of compound **2** with alkyl dihalides to afford terminal bromo substituted ether derivatives (**7a**–**f**). These bromo ether derivatives have been reacted with methyl thioglycolate, 1-methyl piperazine and morpholine to yield **8a**–**f**, **9a**–**f** and **10a**–**f**, respectively, with different carbon chain length spacers (Scheme 1).

All 24 compounds (**7a**–**f**, **8a**–**f**, **9a**–**f** and **10a**–**f**) were evaluated for antimycobacterial activity against four different mycobacterium (*M. tuberculosis* ATCC27294, *M. avium* ATCC 49601 and *M. intracellulare* ATCC 13950) species at a concentration of 50 and 25 μg by diffusion assay. Compounds **7f**,[16] **8e**,[17] **9a**[18] and **9c** demonstrated good to mild inhibition of the mycobacterium cultures. The active compounds were then assayed for determination of minimum inhibitory concentration (MIC) against a variety of *M. tuberculosis* clinical isolates (drug sensitive and resistant) in agar dilution assay as per the NCCLS-M24-T2 recommendations. Briefly, 10 serial twofold dilutions of the compound/standard drug were made in DMSO and incorporated into Middlebrook7H10 agar medium. Individual *M. tuberculosis* isolates at concentration of 1×10^7 CFU/mL were spotted (3–5 μL/spot) on to the media plates. The plates were sealed and incubated at 37 °C for 3–4 weeks. Minimum inhibitory concentration (MIC) was recorded as the highest dilution of the compound(s) that completely inhibited the growth.

Compound **8e** having eight methylene spacers and methyl thioglycolate as linker of thiolactone ring was the most active compound with an MIC value of 1.0–4.0 μg/mL against drug sensitive and resistant strain of *M. tuberculosis*. Compound **7f** having 10 methylene spacers and bromo group as linker also showed moderate activity against *M. tuberculosis*. The other two compounds showed poor (MIC > 16.0 μg/mL) activity against sensitive and resistant strains of *M. tuberculosis*. None of the compounds were found to have significant activity against *M. avium* and *M. intracellulare* cultures (Table 1).

In conclusion, a novel series of antimycobacterial compounds have been designed and synthesized that demonstrated significant (compound **8e**) activity against drug sensitive and resistant *M. tuberculosis* cultures. The antimycobacterial activity of compound **8e** was better than isoniazid on drug resistant clinical isolates of *M. tuberculosis*. These findings clearly indicate that thiolactomycin



Scheme 1. Reagents and conditions: (i) anhydrous KH, THF; (ii) $PyHBr_3$, acetic acid; (iii) AcSH, EtN_3, CH_2Cl_2; (iv) KOH, H_2O–EtOH; (v) $Br(CH_2)_nBr$, anhyd K_2CO_3, acetone, reflux; (vi) RH, anhyd K_2CO_3, acetone, reflux.

Table 1. Antimycobacterial activity of thiolactone ring based analogues

Thiolactone analogues	MIC (μg/mL)				
	M. tuberculosis $H_{37}Rv$ *ATCC* 27294	*M. tuberculosis* clinical isolates		*M. avium* ATCC 49601	*M. intracellulare* ATCC 13950
		Sensitive	Resistant		
7a–e	>16.0	>16.0	>16.0	>16.0	>16.0
7f	**4.0**	**4.0–16.0**	**4.0–16.0**	>16.0	>16.0
8a–d	>16.0	>16.0	>16.0	>16.0	>16.0
8e	**1.0**	**1.0–4.0**	**1.0–4.0**	**8.0**	**4.0**
8f	>16.0	>16.0	>16.0	>16.0	>16.0
9a	>16.0	>16.0	>16.0	>16.0	>16.0
9b	>16.0	>16.0	>16.0	>16.0	>16.0
9c	>16.0	>16.0	>16.0	>16.0	>16.0
9d–f	>16.0	>16.0	>16.0	>16.0	>16.0
10a–f	>16.0	>16.0	>16.0	>16.0	>16.0
Isoniazid	0.25	0.125–0.25	8.0–>16.0	>16.0	8.0

analogues containing bromo, methyl thioglycolate and 1-methyl piperazine groups as linkers resulted in good antimycobacterial activity whereas compounds containing morpholine group as linker result in loss of activity.

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16. Selected spectral data for compound **7f**: ^{1}H NMR (200 MHz; $CDCl_3$) δ 1.3 (s, 3H), 1.2–1.5 (m, 10H), 1.6 (d, 3H, J = 6.6 Hz), 1.6–1.9 (m, 3H), 1.8 (s, 3H), 3.4 (t, 2H, J = 6.6 Hz), 4–4.4 (m, 3H); MS (FAB) 365 [M^++1]. Anal. Calcd for $C_{16}H_{27}BrO_2S$: C, 52.89; H, 7.49. Found: C, 52.78; H, 7.44.
17. Selected spectral data for compound **8e**: ^{1}H NMR (200 MHz; $CDCl_3$) δ 1.2–1.5 (m, 11H), 1.6 (d, 3H, J = 6.6 Hz), 1.9 (s, 3H), 2.5 (s, 3H), 3.2 (s, 2H), 3.7 (s, 3H) 4–4.4 (m, 3H); MS (FAB) 361 [M^++1]. Anal. Calcd for $C_{17}H_{28}O_4S$: C, 56.63; H, 7.83. Found: C, 52.54; H, 7.79.
18. Selected spectral data for compound **9a**: ^{1}H NMR (200MHz; $CDCl_3$) δ 1.6 (d, 3H, J = 6.6 Hz), 1.7 (s, 3H), 1.8–2 (m, 2H), 2.2 (s, 3H), 2.4–2.6 (m, 10H) 4–4.4 (m, 3H); MS (FAB) 285 [M^++1]. Anal. Calcd for $C_{14}H_{24}N_2O_2S$: C, 59.12; H, 8.51. Found: C, 59.08; H, 8.48.